Anti-CD3 WT

GATATCAAACTGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGGCCTCAGTGAAGATGTCCT GCAAGACTTCTGGCTACACCTTTACTAGGTACACGATGCACTGGGTAAAACAGAGGCCTGGACA GGGTCTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTC AAGGACAAGGCCACATTGACTACAGACAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCC TGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGA GGAAGTGGAGGTTCAGGTGGAGTCGACGATTCAGCTGACCCAGTCTCCAGCAATCATGTCTG CATCTCCAGGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAGTTACATGAACTG GGAGTCCCTTATCGCTTCAGTGGCAGTGGGTCTGGGACCTCATACTCTCTCACAATCAGCAGCA TGGAGGCTGAAGATGCTGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCGG CTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAAGTGGAGGTTCTGGT GTACCAGCAGAAGTCAGGCACCTCCCCCAAAAGATGGATTTATGACACATCCAAAGTGGCTTCT TGCTGGGACCAAGCTGGAGCTGAAA

AA Sequence

KDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSG DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKF GSGGSGGVDDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVAS GVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELK

VH2

GYTNYAQKLQGRVTMTTDTSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWG DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSR QGTTVTVSS

VH3

GYTNYAQKLQGRVTMTTDTSTSTAYLQMNSLKTEDTAVYYCARYYDDHYCLDYWG DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSR QGTTVTVSS

VH5

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSR GYTNYADSVKGRFTITTDKSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWG QGTTVTVSS

VH7

GYTNYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCARYYDDHYCLDYWG DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSR QGTTVTVSS

Fig. 2 A (cont.)

SKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGG DIQMTQSPSSLSASVGDRVTITCRASQSVSYMNWYQQKPGKAPKRWIYDT TKVEIK

いち

SKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGG DIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPGKAPKRWIY TKVEIK

$\mathbf{E}\mathbf{I}\mathbf{A}$

SKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGG DIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPGKAPKRWIYDT TKVEIK

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VH2

CTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCACAGAAGTTGCAGGGGC CGCGTCACAATGACTACAGACACTTCCACCAGCACAGCCTACATGGAACTGAGCAGCCTGCGTTCT SAGGACACTGCAACCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGC SACGTCCAACTGGTGCAGTCAGGGGGCTGAAGTGAAAAACCTGGGGGCCTCAGTGAAGGTGTCTG CAAGGCACCACGGTCACCGTCTCCTCA

TH 3

CGCGTCACAATGACTACAGACACTTCCACCAGCACAGCCTACCTGCAAATGAACAGCCTGAAAACT SAGGACACTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGC CTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCACAGAAGTTGCAGGGGC SACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAAACCTGGGGCCTCAGTGAAGGTGTCCTGC

VH5

SACGTCCAACTGGTGCAGTCAGGGGCCTGAAGTGAAAAAAACCTGGGGGCCTCAGTGAAGGTGTCCTGC CTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCAGACAGCGTCAAGGGGC CGCTTCACAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAACTGAGCAGCCTGCGTTCT SAGGACACTGCAACCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGG CAAGGCACCACGGTCACCGTCTCCTCA

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Fig. 2 B (cont.)

VH7

CTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGAC GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACCTGGGGCCTCAGTGAAGGTGTCCTGC CGCGTCACAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAACTGAGCAGCCTGCGTTCT GAGGACACTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGC

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Fig. 2 B (cont.

GGGACCGACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA TGCAGAGCCAGTCAAAGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAAGGCACCCAAA AGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCT CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

717

GACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGTCTCCAGGGGAGCGTGCCACCCTGAGC TGCAGAGCCAGTCAAAGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAAGGCACCCAAA AGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCT GGGACCGACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

71.3

GACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGTCTCCAGGGGAGCGTGCCACCCTGACC TGCAGAGCCAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAAGGCACCCAAA AGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCT GGGACCGACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTGGAGATCAAA Fig. 2 C

vH CDR1

Wt anti-CD3

VH2,3

VH5,7

GYTFTRYTMH

GYTATRYTMH

GYTFTRYTMH

vH CDR2

WT anti-CD3,

VH7

VH5

VH2, 3

YINPSRGYTNYNQKFKD

YINPSRGYTNYADSVKG

YINPSRGYTNYAQKLQG

vH CDR3

WT anti-CD3,

VH2, 3, 5,

YYDDHYCLDY

vK CDR1

WT anti-CD3,

VL3

VL1, 2

RASSSVSYMN

RASQSVSYMN

vK CDR2

WT anti-CD3,

VL1, 2, 3

DTSKVAS

vK CDR3

WT anti-CD3,

VL1, 2, 3

QQWSSNPLT

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Fig. 2 D

vH CDR1

WT anti-CD3 GGCTACACCTTTACTAGGTACACGATG

CAC

VH2, 3 GGCTACACCGCTACTAGGTACACGATG

CAC

VH5,7 GGCTACACCTTTACTAGGTACACGATG

CAC.

vH CDR2

WT anti-CD3,

VH7 TACATTAATCCTAGCCGTGGTTATACT

AATTACAATCAGAAGTTCAAGGAC

VH5 TACATTAATCCTAGCCGTGGTTATACT

AATTACGCAGACAGCGTCAAGGGC

VH2,3 TACATTAATCCTAGCCGTGGTTATACT

AATTACGCACAGAAGTTGCAGGGC

VH CDR3

WT anti-CD3,

VH2, 3,

VH5, 7 TATTATGATGATCATTACTGCCTT

GACTAC

Fig. 2 D (cont.)

vK CDR1

WT anti-CD3,

VL3

AGAGCCAGTTCAAGTGTAAGTTACATG

AAC

VL1, 2

AGAGCCAGTCAAAGTGTAAGTTACATG

AAC

vK CDR2

WT anti-CD3,

VL1-3

ACACATCCAAAGTGGCTTCT

VK CDR3

WT anti-CD3,

VL1-3

CAACAGTGGAGTAGTAACCCGCTCACG

A) anti-CD3 (VH2/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCACAGAAGTTGCAGGGCCGCGTCA CAATGACTACAGACACTTCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAACCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

B) anti-CD3 (VH2/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAP GQGLEWIGYINPSRGYTNYAQKLQGRVTMTTDTSTSTAYME LSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSSGEGT STGSGGSGGGGADDIQMTQSPSSLSASVGDRVTITCRASQ SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

C) anti-CD3 (VH2/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCGCTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCA-CAGAAGTTGCAGGGCCGCGTCACAATGACTACAGA-CACTTCCACCAGCACAGCCTACATGGAACTGAG-CAGCCTGCGTTCTGAGGACACTGCAACCTATTACTGTGCAA GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-TAGTACTGGTTGGTGGAAGTGGAGGTTCAGGTGGAGCA-GACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCT GTCTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGT-CAAAGTGTAAGTTACATGAACTGGTACCAGCA-GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA-CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGAC-CAAGGTGGAGATCAAA

D) anti-CD3 (VH2/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-AQKLQGRVTMTTDTSTSTAYMELSSLRSEDTATYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

E) anti-CD3 (VH2/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCGCTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCA-CAGAAGTTGCAGGGCCGCGTCACAATGACTACAGA-CACTTCCACCAGCACAGCCTACATGGAACTGAG-CAGCCTGCGTTCTGAGGACACTGCAACCTATTACTGTGCAA GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCA-GACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCT GTCTCCAGGGGAGCGTGCCACCCTGACCTGCAGAGC-CAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCA-GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA-CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGAC-CAAGGTGGAGATCAAA

F) anti-CD3 (VH2/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-AQKLQGRVTMTTDTSTSTAYMELSSLRSEDTATYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCOOWSSNPLTFGGGTKVEIK

A) anti-CD3 (VH3/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCACAGAAGTTGCAGGGCCGCGTCA CAATGACTACAGACACTTCCACCAGCACAGCCTACCTGCAA ATGAACAGCCTGAAAACTGAGGACACTGCAGTCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

B) anti-CD3 (VH3/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-

AQKLQGRVTMTTDTSTSTAYLQMNSLKTEDTAVYYCARYYDD-HYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIQMTQSP SSLSASVGDRVTITCRASQSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

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Figure 4 C) anti-CD3 (VH3/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCACAGAAGTTGCAGGGCCGCGTCA CAATGACTACAGACACTTCCACCAGCACAGCCTACCTGCAA ATGAACAGCCTGAAAACTGAGGACACTGCAGTCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGT CTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

D) anti-CD3 (VH3/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAP GQGLEWIGYINPSRGYTNYAQKLQGRVTMTTDTSTSTAYLQ MNSLKTEDTAVYYCARYYDDHYCLDYWGQGTTVTVSSGEGT STGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQ SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

E) anti-CD3 (VH3/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCGCTACTAGGTACACGATG-CACTGGGTAAGGCAGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCA-CAGAAGTTGCAGGGCCGCGTCACAATGACTACAGA-CACTTCCACCAGCACAGCCTACCTGCAAATGAACAGCCT-GAAAACTGAGGACACTGCAGTCTATTACTGTGCAAGATATT ATGATGATCATTACTGCCTTGACTACTGGGGCCAAGGCAC-CACGGTCACCGTCTCCTCAGGCGAAGGTACTAG-TACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGAC-GACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGTC TCCAGGGGAGCGTGCCACCCTGACCTGCAGAGCCAGTT-CAAGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGG-CAAGGCACCCAAAAGATGGATTTATGACACATCCA-AAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGG TCTGGGACCGACTACTCTCTCACAATCAACAGCTTG-GAGGCTGAAGATGCTGCCACTTATTACTGCCAACAGTG-GAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTG-GAGATCAAA

F) anti-CD3 (VH3/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-AQKLQGRVTMTTDTSTSTAYLQMNSLKTEDTAVYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

A) CD3 (VH5/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCAGACAGCGTCAAGGGCCGCTTCA CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAACCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

B) CD3 (VH5/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAP GQGLEWIGYINPSRGYTNYADSVKGRFTITTDKSTSTAYME LSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSSGEGT STGSGGSGGSGGADDIQMTQSPSSLSASVGDRVTITCRASQ SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

C) anti-CD3 (VH5/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCAGACAGCGTCAAGGGCCGCTTCA CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAACCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGT CTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

D) anti-CD3 (VH5/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAP GQGLEWIGYINPSRGYTNYADSVKGRFTITTDKSTSTAYME LSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSSGEGT STGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQ SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

E) anti-CD3 (VH5/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCTTTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACG-CAGACAGCGTCAAGGGCCGCTTCACAATCACTACAGACA-AATCCACCAGCACAGCCTACATGGAACTGAG-CAGCCTGCGTTCTGAGGACACTGCAACCTATTACTGTGCAA GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAG-CAGACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCT CTGTCTCCAGGGGAGCGTGCCACCCTGACCTGCAGAGC-CAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCA-GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGC-CAACAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGG-GACCAAGGTGGAGATCAAA

F) anti-CD3 (VH5/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-ADSVKGRFTITTDKSTSTAYMELSSLRSEDTATYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

A) anti-CD3 (VH7/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACAATCAGAAGTTCAAGGACCGCGTCA CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAGTCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

B) anti-CD3 (VH7/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-QAPGQGLEWIGYINPSRGYT-NYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIQMTQSPSSLSASVGDRVTITCRASQSVSYMNWYQQKPG-

KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCOOWSSNPLTFGGGTKVEIK

C) anti-CD3 (VH7/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACAATCAGAAGTTCAAGGACCGCGTCA CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAGTCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGT CTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

D) anti-CD3 (VH7/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-QAPGQGLEWIGYINPSRGYT-NYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCOOWSSNPLTFGGGTKVEIK

E) anti-CD3 (VH7/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCTTTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACAAT-CAGAAGTTCAAGGACCGCGTCACAATCACTACAGACA-AATCCACCAGCACAGCCTACATGGAACTGAG-CAGCCTGCGTTCTGAGGACACTGCAGTCTATTACTGTGCAA GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCA-GACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCT GTCTCCAGGGGAGCGTGCCACCCTGACCTGCAGAGC-CAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCA-GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA-CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGAC-CAAGGTGGAGATCAAA

F) anti-CD3 (VH7/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-QAPGQGLEWIGYINPSRGYT-NYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQOWSSNPLTFGGGTKVEIK

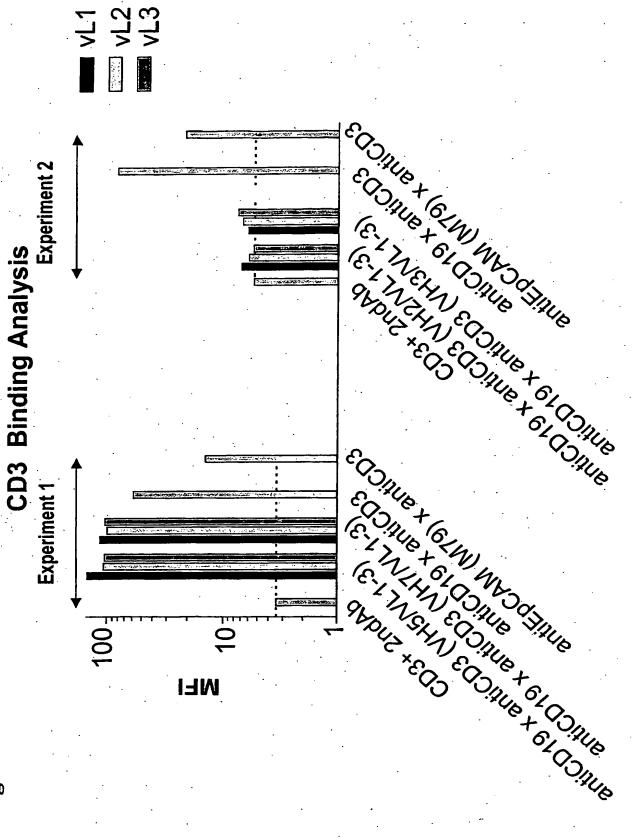


Figure 7.

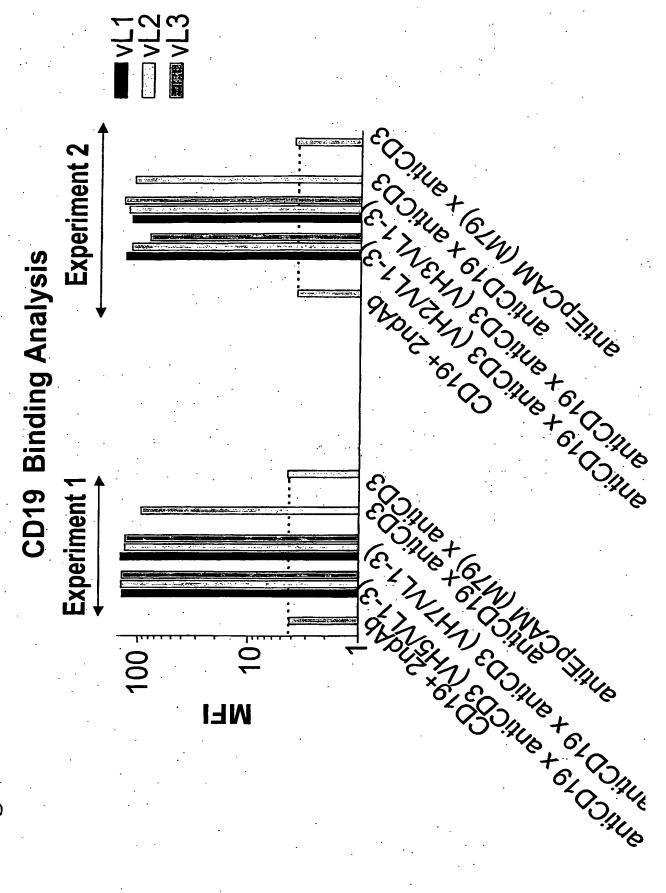


Figure 7

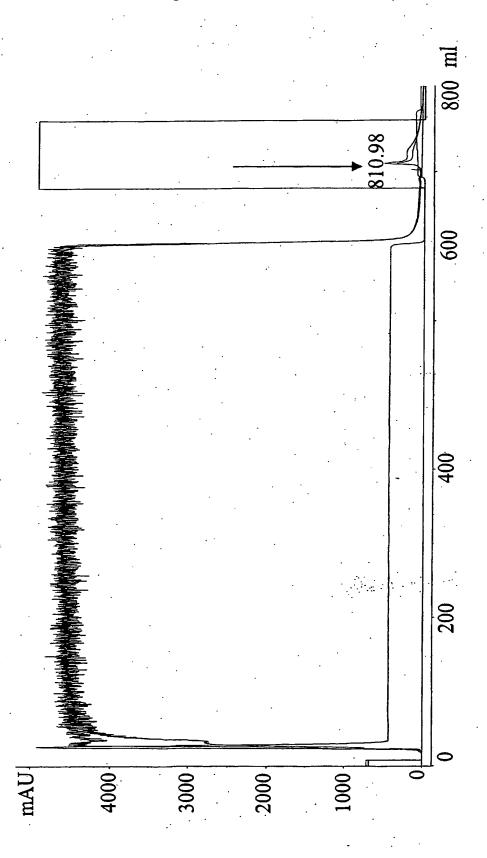


Figure 8

Figure 9

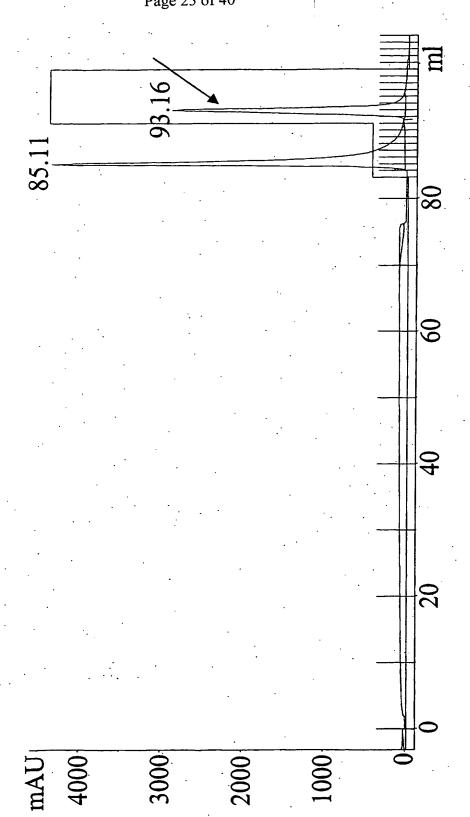
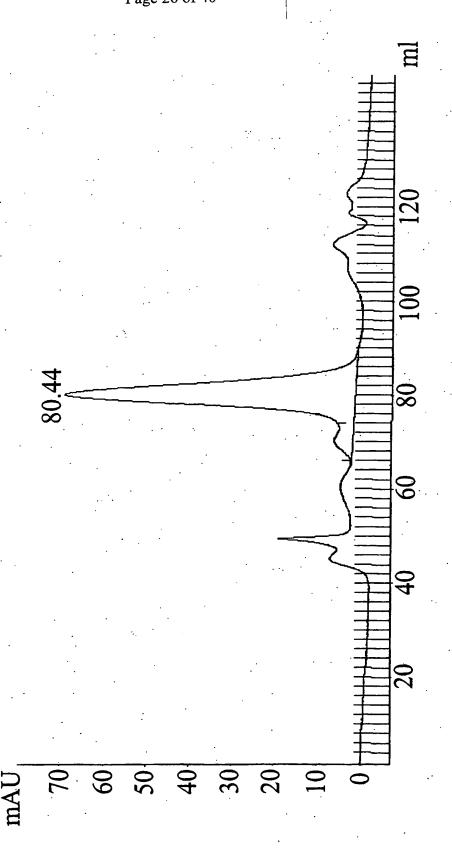


Figure 10



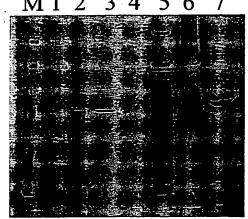
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Figure 11

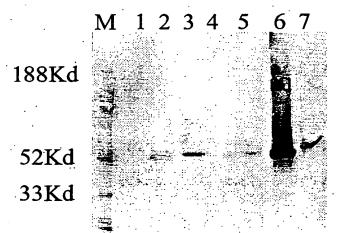
188KD

52 Kd

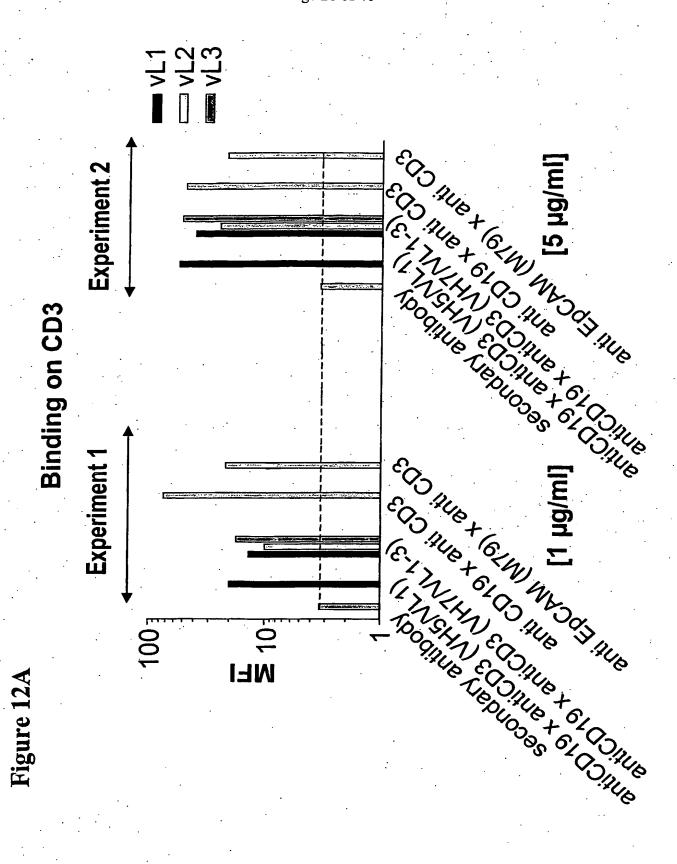
33Kd

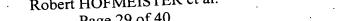


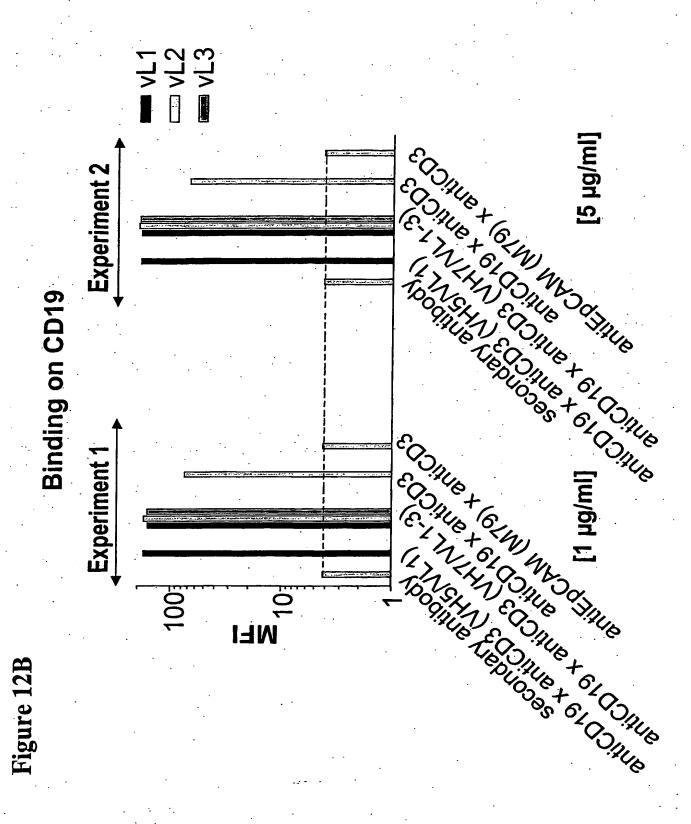
B)











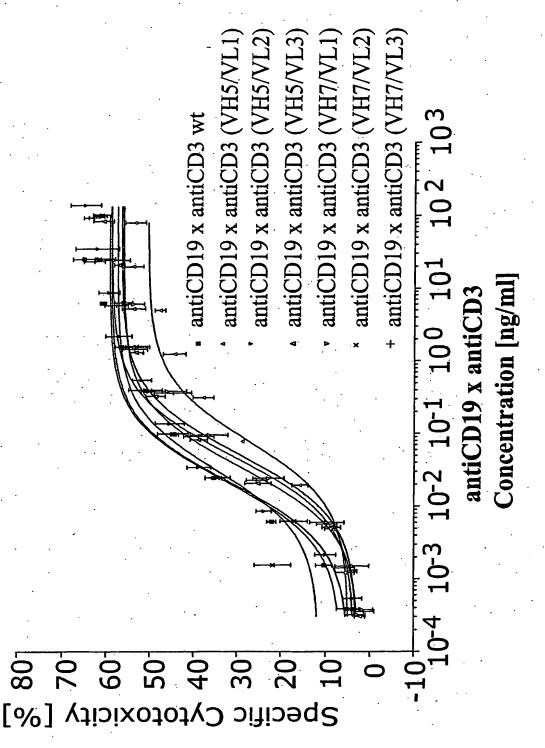


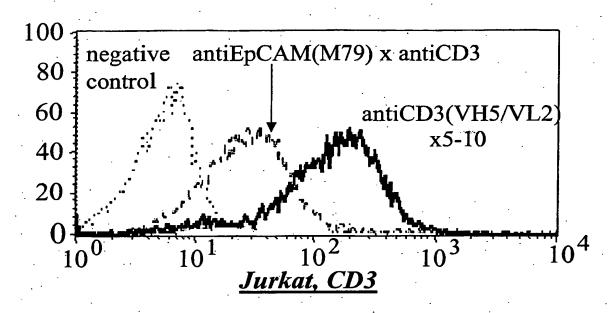
Figure 13

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anti-co3	ო	DIKLOO	SGAELAE	apgas	VKMSCF	TSGYT	FTRYTM	HWVKORP	GQGLEWIGY	DIKLOOSGAELARPGASVKMSCKTSGYTFTRYTMHWVKORPGOGLEWIGYINPSRGYTNYNOKFF
anti-CD3 VH5	VHS	DVQLVQ	SGAEVKE	PGAS	VKVSCE	ASGYT	FTRYTM	HWVRQAP	GOGLEWIGY	DVQLVQSGAEVKKPGASVKVSCKASGYTETTRYTMHWVRQAPGQGLEWIGYINPSRGYTNYADSVF
anti-CD3 VH7	VH7	DVQLVQ	SGAEVKE	PGAS	VKVSCE	ASGYT	FTRYTM	HWVRQAP	GOGLEWIGY	DVQLVQSGAEVKKPGASVKVSCKASGYTETRYTMHWVRQAPGQGLEWIGYINPSRGYTNYNQKFF
anti-CD3 VH2	VH2	DVQLVQS	SGAEVKE	PGAS	VKVSCE	ASGYT.	ATRYTM	HWVRQAP	GQGLEWIGY	DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSRGYTNYAQKL(
anti-CD3	VH3	DVQLVQ	SGAEVKE	PGAS	VKVSCF	ASGYT.	ATRYTM	HWVRQAP	GOGLEWIGY	DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYHNPSRGYTNYAQKLÇ
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anti-CD3	D3	KATLTTDE	KESSTAY	STOW	SLISE	SAVYY	CARYYD	рнусььу	KATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS	SS
anti-CD3	VHS	RETITIDE	KSTSTAY	MELS	SLRSEL)TATYY(CARYYD	DHYCLDY	anti-CD3 VH5 RETITTDKSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSS	SS
anti-co3	VH7	RVTLTTDE	KSTSTAY	MELS	SLRSEL)TAVYY(CARYYD	DHYCLDY	anti-CD3 VH7 RVTITTDKSTSTAYMELSSLRSEDTAVYYCARYYDDHYCLDYWGQGTTVTVSS	SS
anti-co3	VH2	RVTMTTDI	FETSTAY	MELS	SLRSEL)TATY!	CARYYD	DHYCLDY	anti-CD3 VH2 RVTMTTDTSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSS	SS
anti-co3	VH3	RVTMTTD	reteta	(LOMN	SLKTEL)TAVYY(CARYYDI	рнустру	anti-CD3 VH3 RVTMTTDTSTAYLQMNSLKTEDTAVYYCARYYDDHYCLDYWGQGTTVTVSS	SS

Figure 15 A

antiCD3(VH5/VL2) x 5-10 (SEQ ID NO: 37)



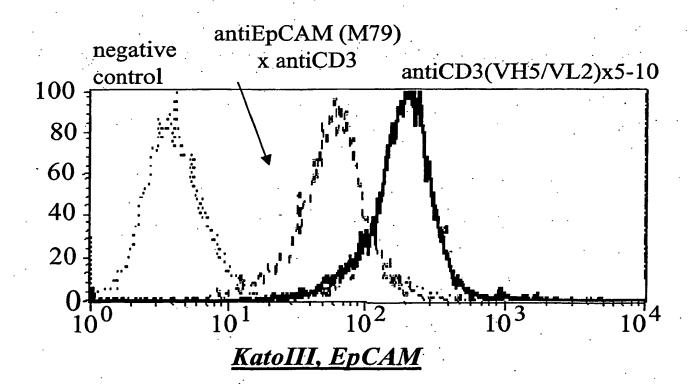
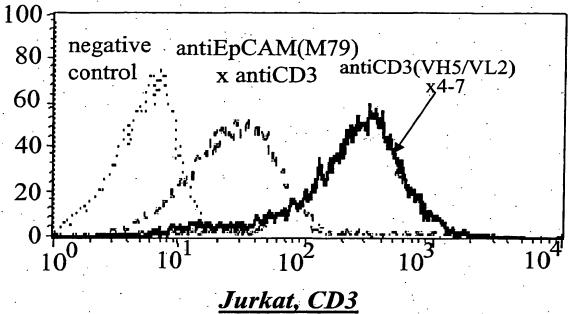


Figure 15B

antiCD3(VH5/VL2) x 4-7 (SEQ ID NO:33)





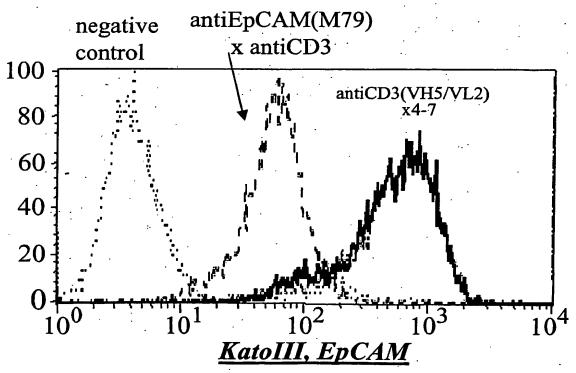
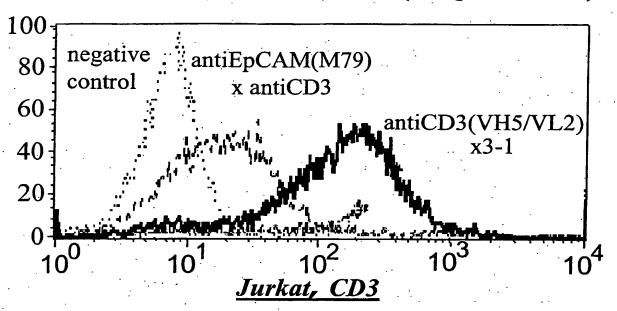


Figure 15C

antiCD3(VH5/VL2) x 3-1 (SEQ ID NO:31)



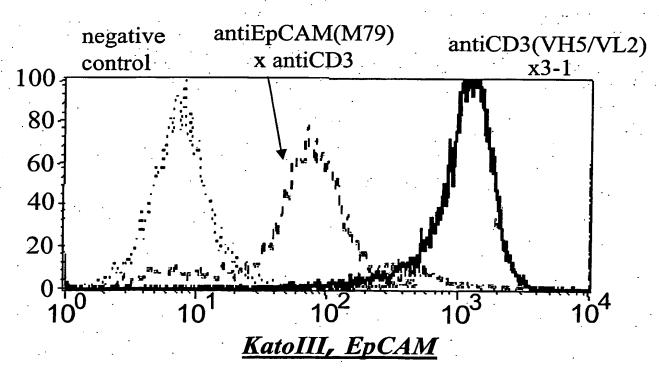
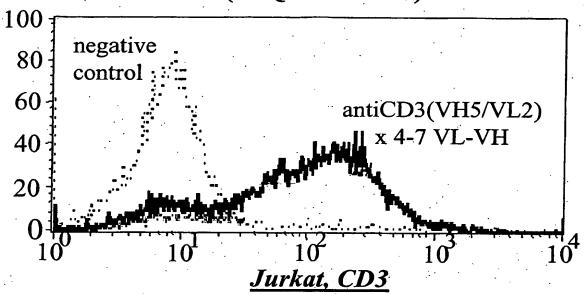


Figure 15 D

antiCD3(VH5/VL2) x 4-7 VL-VH (SEQ ID NO: 35)



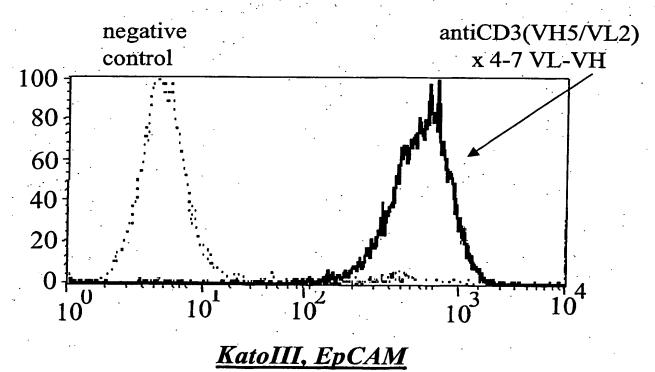
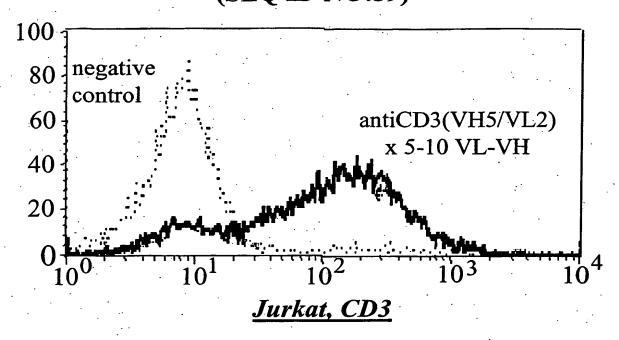
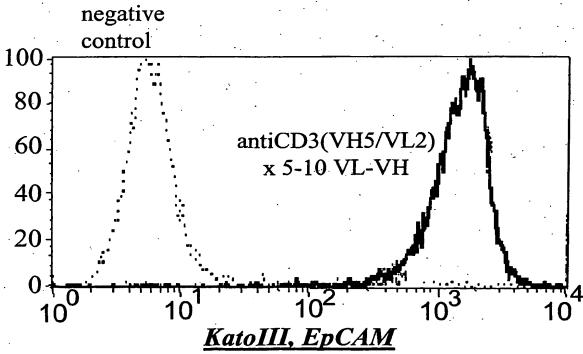


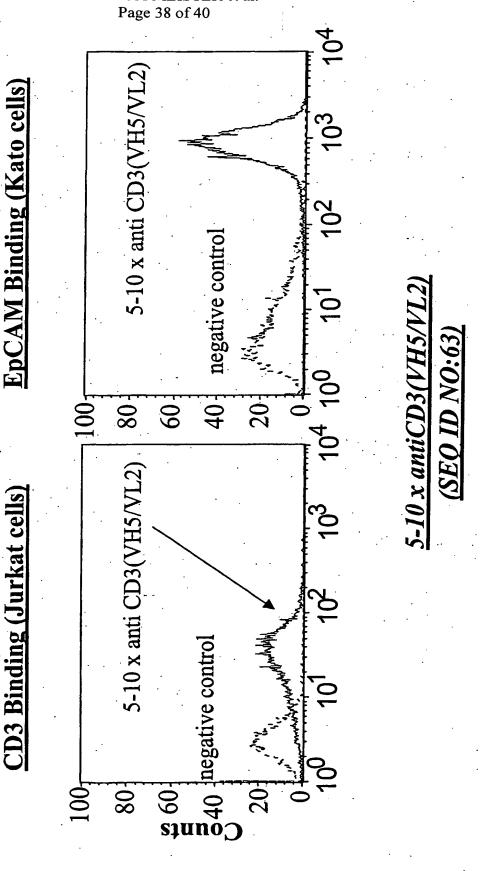
Figure 15 E antiCD3(VH5/VL2) x 5-10 VL-VH (SEQ ID NO:39)



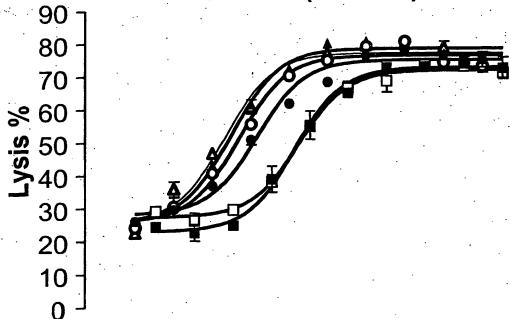


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Figure 16 B

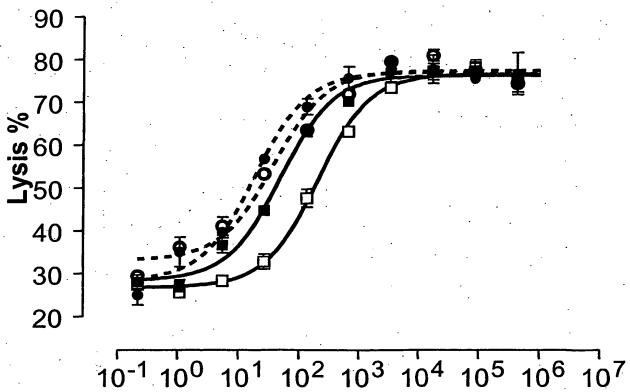


- wt antiCD3 x 3-1
- □ di antiCD3(VH5/VL2) x 3-1
- wt antiCD3 x 5-10
- O di antiCD3 (VH5/VL2) x 5-10
- ▲ wt antiCD3 x 4-7
- △ di antiCD3(VH5/VL2) x 4-7



10⁻¹ 10⁰ 10¹ 10² 10³ 10⁴ 10⁵ 10⁶ 10⁷ bispecific construct [pg/ml]

- 3-1 x antiCD3
- □ 3-1 x antiCD3(VH5/VL2)
- 5-10 x antiCD3
- o 5-10 x antiCD3(VH5/VL2)



 $10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5} 10^{6} 10^{7}$ bispecific construct [pg/ml]